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(54) Title: NUCLEIC ACID MOLECULES ENCODING KAPPA3 OPIOID RECEPTORS, RECEPTORS ENCODED THEREBY, AND USES THEREOF

(57) Abstract

The subject invention provides recombinant nucleic acid molecule which encodes a kappa3 opioid receptor, and the receptor encoded thereby. The subject invention further provides related anti-sense oligonucleotide molecules. The subject invention further provides a related host vector system, and method for using same. The subject invention further provides polyclonal and monoclonal antibodies capable of specifically binding to kappa3 opioid receptor, methods for obtaining same, and methods of using same to detect the presence of, and quantitatively determine the amount of kappa3 opioid receptor in a sample, image and quantitatively determine the amount of cell membrane-bound kappa3 opioid receptor present in a subject, determine a subject's potential sensitivity to a kappa3 opioid receptor-specific agent, determine the affinity of an agent for kappa3 opioid receptor, and identify agonists and antagonists of kappa3 opioid receptor.

NUCLEIC ACID MOLECULES ENCODING KAPPA OPIOID RECEPTORS, RECEPTORS ENCODED THEREBY, AND USES THEREOF

5

10

Background of the Invention

Throughout this application, various publications are referenced. Full citations for these references may be
15 found at the end of the specification immediately preceding the claims. The disclosure of these publications is hereby incorporated by reference into this application to describe more fully the art to which this invention pertains.

20 Opioid drugs have various effects on perception of pain, consciousness, motor control, mood, and autonomic function and can also induce physical dependence (Koob, et al (1992)). The endogenous opioid system plays an important role in modulating endocrine, cardiovascular, respiratory,
25 gastrointestinal and immune functions (Olson, et al (1989)). Opioids exert their actions by binding to specific membrane-associated receptors located throughout the central and peripheral nervous system (Pert, et al. (1973)). The endogenous ligands of these opioid receptors have been
30 identified as a family of more than 20 opioid peptides that derive from the three precursor proteins proopio-melanocortin, proenkephalin, and prodynorphin (Hughes, et al. (1975); Akil, et al. (1984)). Although the opioid peptides belong to a class of molecules distinct from the
35 opioid alkaloids, they share common structural features including a positive charge juxtaposed with an aromatic ring

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that is required for interaction with the receptor (Bradbury, et al. (1976)).

Pharmacological studies have suggested that there are at
5   least three major classes of opioid receptors, designated δ, κ, μ and σ (Simon 1991; Lutz, et al. (1992)). The classes differ in their affinity for various opioid ligands and in their cellular distribution. The different classes of opioid receptors are believed to serve different
10 physiological functions (Olson, et al., (1989); Simon (1991); Lutz & Pfister (1992)). However, there is substantial overlap of function as well as of distribution.

Despite pharmacological and physiological heterogeneity, at
15 least some types of opioid receptors inhibit adenylate cyclase, increase K_+ conductance, and inactivate Ca^{2+} channels through a pertussis toxin-sensitive mechanism (Puttfarcken, et al. 1988; Attali, et al. 1989; Hsia, et al., 1984). These results and others suggest that opioid
20 receptors belong to the large family of cell surface receptors that signal through G proteins (Di Chiara, et al. (1992); Loh, et al. (1990)).

Several attempts to clone cDNAs encoding opioid receptors
25 have been reported. A cDNA encoding an opioid-binding protein (OBCAM) with μ selectivity was isolated (Schofield, et al. (1989)), but the predicted protein lacks transmembrane domains presumed necessary for signal transduction. More recently, the isolation of another cDNA
30 was reported, which was obtained by expression cloning (Xie, et al. (1992)). The deduced protein sequence displays seven putative transmembrane domains and is very similar to the human neuromedin K receptor. However, the affinity of opioid ligands for this receptor expressed in COS cells is

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two orders of magnitude below the expected value, and no subtype selectivity can be shown.

Many cell surface receptor/transmembrane systems consist of
5 at least three membrane-bound polypeptide components: (a) a cell-surface receptor; (b) an effector, such as an ion channel or the enzyme adenylate cyclase; and (c) a guanine nucleotide-binding regulatory polypeptide or G protein, that is coupled to both the receptor and its effector.
10

G protein-coupled receptors mediate the actions of extracellular signals as diverse as light, odorants, peptide hormones and neurotransmitters. Such receptors have been identified in organisms as evolutionarily divergent as yeast
15 and man. Nearly all G protein coupled receptors bear sequence similarities with one another, and it is thought that all share a similar topological motif consisting of seven hydrophobic (and potentially α-helical) segments that span the lipid bilayer (Dohlman et al. (1987); Dohlman et
20 al. (1991)).

G proteins consist of three tightly associated subunits, α, β and γ (1:1:1) in order of decreasing mass. Following agonist binding to the receptor, a conformational change is
25 transmitted to the G protein, which causes the Gα-subunit to exchange a bound GDP for GTP and to dissociate from the $\beta\gamma$-subunits. The GTP-bound form of the α-subunit is typically the effector-modulating moiety. Signal amplification results from the ability of a single receptor to activate
30 many G protein molecules, and from the stimulation by Gα-GTP of many catalytic cycles of the effector.

The family of regulatory G proteins comprises a multiplicity of different α-subunits (greater than twenty in humans),
35 which associate with a smaller pool of β- and γ-subunits

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(greater than four each) (Strothman and Simon (1991)).
Thus, it is anticipated that differences in the α-subunits
probably distinguish the various G protein oligomers,
although the targeting or function of the various α-subunits
5 might also depend on the $\beta\gamma$ subunits with which they
associate (Strothman and Simon (1991).

Improvements in cell culture and in pharmacological methods,
and more recently, use of molecular cloning and gene
10 expression techniques have led to the identification and
characterization of many seven-transmembrane segment
receptors, including new sub-types and sub-sub-types of
previously identified receptors. The α_1 and α_2-adrenergic
receptors once thought to each consist of single receptor
15 species, are now known to each be encoded by at least three
distinct genes (Kobilka et al. (1987); Regan et al. (1988);
Cotecchia et al. (1988); Lomasney (1990)). In addition to
rhodopsin in rod cells, which mediates vision in dim light,
three highly similar cone pigments mediating color vision
20 have been cloned (Nathans et al. (1986)A; and Nathans et al.
(1986)B). All of the family of G protein-coupled receptors
appear to be similar to other members of the family of G
protein-coupled receptors (e.g., dopaminergic, muscarinic,
serotonergic, tachykinin), and each appears to share the
25 characteristic seven-transmembrane segment topography.

When comparing the seven-transmembrane segment receptors
with one another, a discernible pattern of amino acid
sequence conservation is observed. Transmembrane domains
30 are often the most similar, whereas the amino and carboxyl
terminal regions and the cytoplasmic loop connecting
transmembrane segments V and VI can be quite divergent
(Dohlman et al. (1987)).

Interaction with cytoplasmic polypeptides, such as kinases and G proteins, was predicted to involve the hydrophobic loops connecting the transmembrane domains of the receptor. The challenge, however, has been to determine which features

5   are preserved among the seven-transmembrane segment receptors because of conservation of function, and which divergent features represent structural adaptations to new functions. A number of strategies have been used to test these ideas, including the use of recombinant DNA and gene

10   expression techniques for the construction of substitution and deletion mutants, as well as of hybrid or chimeric receptors (Dohlman et al. (1991)).

With the growing number of receptor sub-types, G-protein

15   subunits, and effectors, characterization of ligand binding and G protein recognition properties of these receptors is an important area for investigation. It has long been known that multiple receptors can couple to a single G protein and, as in the case of epinephrine binding to β_2- and α_2-

20   adrenergic receptors, a single ligand can bind to multiple functionally distinct receptor sub-types. Moreover, G proteins with similar receptor and effector coupling specificities have also been identified. For example, three species of human G_i have been cloned (Itoh et al. (1988)),

25   and alternate mRNA splicing has been shown to result in multiple variants of G_s (Kozasa et al. (1988)). Cloning and over production of the muscarinic and α_2-adrenergic receptors led to the demonstration that a single receptor sub-type, when expressed at high levels in the cell, will

30   couple to more than one type of G protein.

Opioid receptors are known to be sensitive to reducing agents, and the occurrence of a disulfide bridge has been postulated as essential for ligand binding (Gioannini, et

al. (1989)). For rhodopsin, muscarinic, and ß-adrenergic receptors, two conserved cysteine residues in each of the two first extracellular loops have been shown critical for stabilizing the functional protein structure and are
5   presumed to do so by forming a disulfide bridge. Structure/function studies of opioid ligands have shown the importance of a protonated amine group for binding to the receptor with high affinity. The binding site of the receptor might, therefore, possess a critical negatively
10   charged counterpart. Catecholamine receptors display in their sequence a conserved aspartate residue that has been shown necessary for binding the positively charged amine group of their ligands.

15   Given the complexity and apparent degeneracy of function of various opioid receptors, a question of fundamental importance is how, and under what circumstances do specific sub-type and sub-sub-type receptors exert their physiological effect in the presence of the appropriate
20   stimulatory ligand. A traditional approach to answering this question has been to reconstitute the purified receptor and G protein components _in vitro_. Unfortunately, purification schemes have been successful for only a very limited number of receptor sub-types and their cognate G-
25   proteins. Alternatively, heterologous expression systems can be of more general usefulness in the characterization of cloned receptors and in elucidating receptor G protein coupling specificity (Marullo et al. (1988); Payette et al. (1990); King et al. (1990)).
30
One such system was recently developed in yeast cells, in which the genes for a mammalian β_2-adrenergic receptor and G, α-subunit were coexpressed (King et al. 1990). Expression of the β_2-adrenergic receptor to levels several

hundred-fold higher than in any human tissue was attained, and ligand binding was shown to be of the appropriate affinity, specificity, and stereoselectivity. Moreover, a β_2-adrenergic receptor-mediated activation of the pheromone

5   signal transduction pathway was demonstrated by several criteria, including imposition of growth arrest, morphological changes, and induction of a pheromone-responsive promoter (FUS1) fused to the Escherichia coli lacz gene (encoding β-galactosidase) (King et al. 1990).

10

Finally, expression of a single receptor in the absence of other related sub-types is often impossible to achieve, even in isolated, non-recombinant mammalian cells. Thus, there has been considerable difficulty in applying the standard

15 approaches of classical genetics or even the powerful techniques of molecular biology to the study of opioid receptors. In particular, means are needed for the identification of the DNA sequences encoding individual opioid receptors. Given such isolated, recombinant

20 sequences, it is possible to address the heretofore intractable problems associated with design and testing of isoform-specific opioid receptor agonists and antagonists. The availability of cDNAs encoding the opioid receptors will permit detailed studies of signal-transduction mechanisms

25 and reveal the anatomical distribution of the mRNAs of these receptors, providing information on their expression pattern in the nervous system. This information should ultimately allow better understanding of the opioid system in analgesia, and also the design of more specific therapeutic

30 drugs.

Availability of polynucleotide sequences encoding opioid receptors, and the polypeptide sequences of the encoded receptors, will significantly increase the capability to

8

design pharmaceutical compositions, such as analgesics, with enhanced specificity of function. In general, the availability of these polypeptide sequences will enable efficient screening of candidate compositions. The
5 principle in operation through the screening process is straightforward: natural agonists and antagonists bind to cell-surface receptors and channels to produce physiological effects; certain other molecules bind to receptors and channels; therefore, certain other molecules may produce
10 physiological effects and act as therapeutic pharmaceutical agents. Thus, the ability of candidate drugs to bind to opioid receptors can function as an extremely effective screening criterion for the selection of pharmaceutical compositions with a desired functional efficacy.
15

Prior methods for screening candidate drug compositions based on their ability to preferentially bind to cell-surface receptors has been limited to tissue-based techniques. In these techniques, animal tissues rich in the
20 receptor type of interest are extracted and prepared; candidate drugs are then allowed to interact with the prepared tissue and those found to bind to the receptors are selected for further study. However, these tissue-based screening techniques suffer from several significant
25 disadvantages. First, they are expensive because the source of receptor cell tissue--laboratory animals--is expensive. Second, extensive technical input is required to operate the screens. And, third, the screens may confuse the results because there are no tissues where only one receptor subtype
30 is expressed exclusively. With traditional prior art screens, the wrong interactions are observed or, at best, the proper interactions are observed together with unwanted interactions.

The nucleic acid molecules, proteins and methods of the subject invention overcome the difficulties discussed _supra_ in connection with kappa₃ opioid receptors.

Summary of the Invention

The subject invention provides recombinant nucleic acid
molecule which encodes a $kappa_3$ opioid receptor.

The subject invention further provides an anti-sense
oligonucleotide molecule capable of specifically hybridizing
to an mRNA molecule encoding $kappa_3$ opioid receptor, at the
portion thereof encoding $kappa_3$ opioid receptor, so as to
prevent translation of the mRNA molecule.

The subject invention further provides a nucleic acid
molecule encoding the anti-sense oligonucleotide molecule of
the subject invention, wherein the nucleic acid molecule is
capable of being expressed in a suitable host cell.

The subject invention further provides a vector comprising
the recombinant nucleic acid molecule of the subject
invention.

The subject invention further provides a host vector system
for the production of a $kappa_3$ opioid receptor which
comprises the vector of the subject invention in a suitable
host cell.

The subject invention further provides a method for
producing a $kappa_3$ opioid receptor which comprises growing
the host vector system of the subject invention under
conditions permitting the production of the $kappa_3$ opioid
receptor, and recovering the $kappa_3$ opioid receptor produced
thereby.

The subject invention further provides the nucleic acid
molecule of the subject invention, wherein the nucleic acid
molecule is labeled with a detectable marker.

5   The subject invention further provides a method for
detecting the presence of kappa$_3$ opioid receptor-encoding
nucleic acid molecules present in a sample which comprises
contacting the sample with the labeled nucleic acid molecule
of the subject invention under conditions permitting the
10   labeled nucleic acid molecule to form a complex with kappa$_3$
opioid receptor-encoding nucleic acid molecules present in
the sample, and detecting the presence of such complex
formed so as to thereby detect the presence of kappa$_3$ opioid
receptor-encoding nucleic acid molecules present in the
15   sample.

The subject invention further provides a method for
quantitatively determining the amount of kappa$_3$ opioid
receptor-encoding nucleic acid molecules present in a
20   sample which comprises contacting the sample with a suitable
amount of the labeled nucleic acid molecule of the subject
invention under conditions permitting the labeled nucleic
acid molecule to form a complex with kappa$_3$ opioid receptor-
encoding nucleic acid molecules present in the sample,
25   quantitatively determining the amount of complex so formed
so as to thereby quantitatively determine the amount of
kappa$_3$ opioid receptor-encoding nucleic acid molecules
present in the sample.

30   The subject invention further provides a purified native
kappa$_3$ opioid receptor.

The subject invention further provides the variant kappa₃ opioid receptor encoded by the recombinant nucleic acid molecule of the subject invention.

5   The subject invention further provides a method for obtaining partially purified polyclonal antibodies capable of specifically binding to kappa₃ opioid receptor and thereby competing with opioid binding thereto which method comprises (a) immunizing a subject with the kappa₃ opioid
10   receptor encoded by the nucleic acid molecule of the subject invention, (b) recovering from the immunized subject serum comprising antibodies capable of specifically binding to kappa₃ opioid receptor and thereby competing with opioid binding thereto, and (c) partially purifying the antibodies
15   present in the serum, thereby obtaining partially purified polyclonal antibodies capable of specifically binding to kappa₃ opioid receptor and thereby competing with opioid binding thereto.

20   The subject invention further provides the partially purified antibodies produced by the method of the subject invention.

The subject invention further provides a method for
25   obtaining a purified monoclonal antibody capable of specifically binding to kappa₃ opioid receptor and thereby competing with opioid binding thereto which method comprises (a) immunizing a subject with the kappa₃ opioid receptor encoded by the nucleic acid molecule of the subject
30   invention, (b) recovering from the immunized subject a B cell-containing cell sample, (c) contacting the B cell-containing cell sample so recovered with myeloma cells under conditions permitting fusion of the myeloma cells with the B cells therein so as to form hybridoma cells, (d) isolating

from the resulting sample a hybridoma cell capable of producing a monoclonal antibody capable of specifically binding to kappa$_3$ opioid receptor and thereby competing with opioid binding thereto, (e) growing the hybridoma cell so

5   isolated under conditions permitting the production of the monoclonal antibody, and (f) recovering the monoclonal antibody so produced, thereby obtaining a purified monoclonal antibody capable of specifically binding to kappa$_3$ opioid receptor and thereby competing with opioid

10   binding thereto.

The subject invention further provides the hybridoma cell produced in step (d) of the subject invention.

15   The subject invention further provides the purified monoclonal antibody produced by the method of the subject invention.

The subject invention further provides an antibody capable

20   of specifically binding to kappa$_3$ opioid receptor and thereby competing with opioid binding thereto, said antibody being labeled with a detectable marker.

The subject invention further provides a method for

25   detecting the presence of kappa$_3$ opioid receptor in a sample which comprises contacting the sample with the antibody of the subject invention under conditions permitting the antibody to form a complex with kappa$_3$ opioid receptor present in the sample, and detecting the presence of complex

30   so formed, so as to thereby detect the presence of kappa$_3$ opioid receptor in the sample.

The subject invention further provides a method for quantitatively determining the amount of kappa$_3$ opioid

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receptor in a sample which comprises contacting the sample
with the antibody of the subject invention under conditions
permitting the antibody to form a complex with kappa₃ opioid
receptor present in the sample, quantitatively determining

5   the amount of complex so formed, and comparing the amount so
determined with a known standard, so as to thereby
quantitatively determine the amount of kappa₃ opioid
receptor in the sample.

10   The subject invention further provides a composition which
comprises the antibody of the subject invention in an amount
effective to permit imaging cell membrane-bound kappa₃
opioid receptor present in a subject, and a pharmaceutically
acceptable carrier.

15
The subject invention further provides a method for imaging
cell membrane-bound kappa₃ opioid receptor present in a
subject which comprises administering to the subject an
amount of the composition of the subject invention effective

20   to permit imaging cell membrane-bound kappa₃ opioid receptor
present in the subject under conditions permitting the
antibody of the composition to specifically bind to cell
membrane-bound kappa₃ opioid receptor present in the
subject, and imaging the antibody specifically bound to cell

25   membrane-bound kappa₃ opioid receptor present in the subject
after a suitable period of time, so as to thereby image cell
membrane-bound kappa₃ opioid receptor present in the
subject.

30   The subject invention further provides a composition which
comprises the antibody of the subject invention in an amount
effective to permit quantitatively determining the amount of
cell membrane-bound kappa₃ opioid receptor present in a
subject, and a pharmaceutically acceptable carrier.

15

The subject invention further provides a method for quantitatively determining the amount of cell membrane-bound kappa₃ opioid receptor present in a subject which comprises administering to the subject an amount of the composition of

5 the subject invention effective to permit quantitatively determining the amount of cell membrane-bound kappa₃ opioid receptor present in the subject under conditions permitting the antibody of the composition to specifically bind to cell membrane-bound kappa₃ opioid receptor in the subject,

10 quantitatively determining the amount of antibody specifically bound to cell membrane-associated kappa₃ opioid receptors present in the subject, and comparing the amount so determined with a known standard so as to thereby quantitatively determine the amount of cell membrane-bound

15 kappa₃ opioid receptor present in the subject.

The subject invention further provides a method for determining a subject's potential sensitivity to a kappa₃ opioid receptor-specific agent which comprises

20 quantitatively determining the amount of cell membrane-bound kappa₃ opioid receptor present in the subject by the method of the subject invention, and comparing the amount of cell membrane-bound kappa₃ opioid receptor so determined with the amount of cell membrane-bound kappa₃ opioid receptor present

25 in a subject having a known sensitivity to the agent, so as to thereby determine the subject's potential sensitivity to the kappa₃ opioid receptor-specific agent.

The subject invention further provides a method for

30 determining the affinity of an agent for kappa₃ opioid receptor which comprises (a) contacting a predetermined amount of kappa₃ opioid receptor with (i) a predetermined amount of the agent together with (ii) a predetermined amount of a detectable known ligand of kappa₃ opioid

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receptor, said known ligand being a known ligand of kappa$_3$ opioid receptor with a known affinity therefore, under conditions which would permit the formation of a complex between kappa$_3$ opioid receptor and the detectable known ligand in the absence of the agent, (b) quantitatively determining the amount of kappa$_3$ opioid receptor-detectable known ligand complex so formed, (c) comparing the amount of kappa$_3$ opioid receptor-detectable known ligand complex determined in step (b) with the amount of kappa$_3$ opioid receptor-detectable known ligand complex formed in the absence of the agent, and (d) determining the affinity of the agent for kappa$_3$ opioid receptor based on the comparison in step (c).

The subject invention further provides a method for determining the affinity of an agent for kappa$_3$ opioid receptor which comprises (a) contacting kappa$_3$ opioid receptor with the agent under conditions which would permit the formation of a complex between kappa$_3$ opioid receptor and a known ligand thereof, (b) contacting a predetermined amount of the agent-receptor complex with a predetermined amount of the antibody of the subject invention under conditions which would permit the formation of a complex between kappa$_3$ opioid receptor and the antibody in the absence of the agent, (c) quantitatively determining the amount of kappa$_3$ opioid receptor-antibody complex so formed, and (d) determining the affinity of the agent for kappa$_3$ opioid receptor based on the amount of kappa$_3$ opioid receptor-antibody complex determined in step (c).

The subject invention further provides a method for determining whether a known ligand of kappa$_3$ opioid receptor is an agonist thereof which comprises (a) contacting the kappa$_3$ opioid receptor with (i) a predetermined amount of

the ligand and (ii) a predetermined amount of GTP or analog thereof under conditions which would permit the binding of the ligand to the $kappa_3$ opioid receptor in the absence of GTP or analog thereof, (b) quantitatively determining the

5   percentage of such predetermined amount of ligand bound to the $kappa_3$ opioid receptor, and (c) comparing the percentage so determined with the percentage of such predetermined amount of ligand bound to the $kappa_3$ opioid receptor in the absence of GTP or analog thereof, a lower percentage in the

10 presence of GTP or analog thereof relative to the percentage in the absence of GTP or analog thereof indicating that the ligand is an agonist of $kappa_3$ opioid receptor.

Finally, the subject invention provides a method for

15 determining whether a ligand of $kappa_3$ opioid receptor is an antagonist thereof which comprises (a) contacting the $kappa_3$ opioid receptor with (i) a predetermined amount of the ligand and (ii) a predetermined amount of GTP or analog thereof under conditions which would permit the binding of

20 the ligand to the $kappa_3$ opioid receptor in the absence of GTP or analog thereof, (b) quantitatively determining the percentage of such predetermined amount of ligand bound to the $kappa_3$ opioid receptor, and (c) comparing the percentage so determined with the percentage of such predetermined

25 amount of ligand bound to the $kappa_3$ opioid receptor in the absence of GTP or analog thereof, an equality between the percentage in the presence of GTP or analog thereof and the percentage in the absence of GTP or analog thereof indicating that the ligand is an antagonist of $kappa_3$ opioid

30 receptor.

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Brief Description of the Figures

Figure 1

5 Nucleic acid sequence of mouse $kappa_3$ opioid receptor cDNA and predicted amino acid sequence thereof.

Figure 2

Effect of antisense treatment on $kappa_3$ analgesic activity.

10

Detailed Description of the Invention

The plasmid 13-2A4 was deposited pursuant to, and in
5 satisfaction of, the requirements of the Budapest Treaty on
the International Recognition of the Deposit of
Microorganisms for the Purposes of Patent Procedure with the
American Type Culture Collection (ATCC), 12301 Parklawn
Drive, Rockville, Maryland 20852 under ATCC Accession No._
10 75608 . The plasmid 13-2A4 was deposited with the ATCC on
November 5, 1993.

Specifically, the subject invention provides recombinant
nucleic acid molecule which encodes a kappa$_3$ opioid
15 receptor.

As used herein, a recombinant nucleic acid molecule is a
nucleic acid molecule which does not occur as an individual
molecule in nature and which is obtained through the use of
20 recombinant technology.

The designation "kappa$_3$ opioid receptor" includes all opioid
receptors which display the pharmacological behavior
attributed to kappa$_3$ opioid receptors in the art.
25
In one embodiment, the nucleic acid molecule is a DNA
molecule. The DNA molecule may be a cDNA molecule. In
another embodiment, the nucleic acid molecule is an RNA
molecule. The RNA molecule may be an mRNA molecule.
30
In one embodiment, the kappa$_3$ opioid receptor is native
kappa$_3$ opioid receptor. Native kappa$_3$ opioid receptor means
a full length, naturally occurring membrane-bound kappa$_3$
opioid receptor protein.

In another embodiment, the kappa₃ opioid receptor is a variant kappa₃ opioid receptor. As used herein, variant kappa₃ opioid receptor means a protein comprising a portion of kappa₃ opioid receptor, which protein portion is capable

5 of (a) specifically binding to a kappa₃ opioid receptor-specific ligand, and (b) competing with kappa₃ opioid receptor for binding to a kappa₃ opioid receptor-specific ligand. A variant kappa₃ opioid receptor may comprise, by way of example, a fragment of kappa₃ opioid receptor, a

10 point mutant of kappa₃ opioid receptor, a deletion mutant of kappa₃ opioid receptor, or a point and deletion mutant of kappa₃ opioid receptor. The variant kappa₃ opioid receptor may be an aqueous-soluble variant kappa₃ opioid receptor, and may either be glycosylated or non-glycosylated.

15

The kappa₃ opioid receptor may be a mammalian kappa₃ opioid receptor. In one embodiment, the mammalian kappa₃ opioid receptor is a murine kappa₃ opioid receptor. The murine kappa₃ opioid receptor may be the murine kappa₃ opioid

20 receptor having the sequence shown in Figure 1. In another embodiment, the mammalian kappa₃ opioid receptor is a human kappa₃ opioid receptor.

The subject invention further provides an anti-sense

25 oligonucleotide molecule capable of specifically hybridizing to an mRNA molecule encoding kappa₃ opioid receptor, at the portion thereof encoding kappa₃ opioid receptor, so as to prevent translation of the mRNA molecule. Such anti-sense oligonucleotide molecules are discussed in more detail

30 infra.

As used herein, "capable of specifically hybridizing" means capable of binding to an mRNA molecule encoding kappa₃

21

opioid receptor but not capable of binding to an mRNA
molecule encoding a non-kappa₃ opioid receptor protein.

5   The subject invention further provides a nucleic acid
molecule encoding the anti-sense oligonucleotide molecule of
the subject invention, wherein the nucleic acid molecule is
capable of being expressed in a suitable host cell. In one
embodiment, the nucleic acid molecule encoding the anti-
sense oligonucleotide molecule is a DNA molecule.

10

The subject invention further provides a vector comprising
the recombinant nucleic acid molecule of the subject
invention.

15   In accordance with the invention, numerous vector systems
for expression of kappa₃ opioid receptor may be employed.
For example, one class of vectors utilizes DNA elements
which are derived from animal viruses such as bovine
papilloma virus, polyoma virus, adenovirus, vaccinia virus,

20   baculovirus, retroviruses (RSV, MMTV or MoMLV), Semliki
Forest virus or SV40 virus. Additionally, cells which have
stably integrated the DNA into their chromosomes may be
selected by introducing one or more markers which allow for
the selection of transfected host cells. The marker may

25   provide, for example, prototropy to an auxotrophic host,
biocide resistance, (e.g., antibiotics) or resistance to
heavy metals such as copper or the like. The selectable
marker gene can be either directly linked to the DNA
sequences to be expressed, or introduced into the same cell

30   by cotransformation. Additional elements may also be needed
for optimal synthesis of mRNA. These elements may include
splice signals, as well as transcriptional promoters,
enhancers, and termination signals.

In one embodiment, the vector is a plasmid. The plasmid may be the plasmid designated 13-2A4 (ATCC Accession No.75608_).

5 The subject invention further provides a host vector system for the production of a kappa₃ opioid receptor which comprises the vector of the subject invention in a suitable host cell. Methods of making host vector systems are well known to those skilled in the art.

10 The suitable host cell may be a bacterial cell. In one embodiment, the bacterial cell is an E. coli cell.

The suitable host cell may also be an eucaryotic cell. In one embodiment, the eucaryotic cell is a mammalian cell. In 15 the preferred embodiment, the mammalian cell is a COS cell. The COS cell may be a COS-7 cell.

The subject invention further provides a method for producing a kappa₃ opioid receptor which comprises growing 20 the host vector system of the subject invention under conditions permitting the production of the kappa₃ opioid receptor, and recovering the kappa₃ opioid receptor produced thereby.

25 Methods and conditions for growing host vector systems and for recovering the kappa₃ opioid receptor so produced are well known to those skilled in the art, and may be varied or optimized depending upon the specific vector and host cell employed. Such recovery methods include, by way of example, 30 gel electrophoresis, ion exchange chromatography, affinity chromatography or combinations thereof.

The subject invention further provides the nucleic acid molecule of the subject invention, wherein the nucleic acid

molecule is labeled with a detectable marker. In one embodiment, the labeled nucleic acid molecule of the subject invention need not encode a functional receptor, but need only possess sufficient homology to a functional kappa₃ opioid receptor sequence to permit hybridization thereto.

The subject invention further provides a method for detecting the presence of kappa₃ opioid receptor-encoding nucleic acid molecules present in a sample which comprises contacting the sample with the labeled nucleic acid molecule of the subject invention under conditions permitting the labeled nucleic acid molecule to form a complex with kappa₃ opioid receptor-encoding nucleic acid molecules present in the sample, and detecting the presence of such complex formed so as to thereby detect the presence of kappa₃ opioid receptor-encoding nucleic acid molecules present in the sample.

The nucleic acid molecules detected may be DNA or RNA molecules. In one embodiment, the nucleic acid molecules detected are mRNA molecules.

The sample may be, for example, a solution comprising nucleic acid molecules, or an immobilized sample of nucleic acid molecules.

Conditions permitting the labeled nucleic acid molecule to form a complex with kappa₃ opioid receptor-encoding nucleic acid molecules present in the sample, as well as methods for detecting the presence of such complex formed, are well known in the art.

The subject invention further provides a method for quantitatively determining the amount of kappa₃ opioid

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receptor-encoding nucleic acid molecules present in a sample which comprises contacting the sample with a suitable amount of the labeled nucleic acid molecule of the subject invention under conditions permitting the labeled nucleic

5   acid molecule to form a complex with kappa₃ opioid receptor-encoding nucleic acid molecules present in the sample, quantitatively determining the amount of complex so formed so as to thereby quantitatively determine the amount of kappa₃ opioid receptor-encoding nucleic acid molecules

10   present in the sample.

The amount of kappa₃ opioid receptor-encoding nucleic acid molecules present in a sample need not be an absolute number, in the sense that it need not be the actual number

15   of nucleic acid molecules in the sample. Rather, the amount may merely correlate with this number.

The subject invention further provides a purified native kappa₃ opioid receptor. As used herein, "purified" means

20   free of any other opioid receptors. For example, the purified kappa₃ opioid receptor may include membrane fragments, other non-kappa₃ opioid receptor proteins, and a suitable buffer. Alternatively, the purified kappa₃ opioid receptor may include only the receptor, or receptor bound by

25   a membrane, and a suitable buffer.

The subject invention further provides the variant kappa₃ opioid receptor encoded by the recombinant nucleic acid molecule of the subject invention. In one embodiment, the

30   variant kappa₃ opioid receptor is a purified variant kappa₃ opioid receptor.

The subject invention further provides a method for obtaining partially purified polyclonal antibodies capable

of specifically binding to kappa₃ opioid receptor and thereby competing with opioid binding thereto which method comprises (a) immunizing a subject with the kappa₃ opioid receptor encoded by the nucleic acid molecule of the subject

5   invention, (b) recovering from the immunized subject serum comprising antibodies capable of specifically binding to kappa₃ opioid receptor and thereby competing with opioid binding thereto, and (c) partially purifying the antibodies present in the serum, thereby obtaining partially purified

10 polyclonal antibodies capable of specifically binding to kappa₃ opioid receptor and thereby competing with opioid binding thereto.

As used herein, partially purified antibodies means a

15 composition which comprises antibodies which specifically bind to kappa₃ opioid receptor, and consists of fewer protein impurities than does the serum from which the antibodies are derived. A protein impurity means a protein other than the antibodies specific for kappa₃ opioid

20 receptor. For example, the partially purified antibodies might be an IgG preparation.

Methods of recovering serum from a subject are well known to those skilled in the art. Methods of partially purifying

25 antibodies are also well known to those skilled in the art, and include, by way of example, filtration, ion exchange chromatography, and precipitation.

As used herein, "capable of specifically binding to kappa₃

30 opioid receptor and thereby competing with opioid binding thereto" means binding either directly to an opioid-binding site or to a non-opioid-binding site resulting in inhibition of opioid binding to the receptor.

26

The subject invention further provides the partially purified antibodies produced by the method of the subject invention.

5   The subject invention further provides a method for obtaining a purified monoclonal antibody capable of specifically binding to kappa$_3$ opioid receptor and thereby competing with opioid binding thereto which method comprises (a) immunizing a subject with the kappa$_3$ opioid receptor
10 encoded by the nucleic acid molecule of the subject invention, (b) recovering from the immunized subject a B cell-containing cell sample, (c) contacting the B cell-containing cell sample so recovered with myeloma cells under conditions permitting fusion of the myeloma cells with the
15 B cells therein so as to form hybridoma cells, (d) isolating from the resulting sample a hybridoma cell capable of producing a monoclonal antibody capable of specifically binding to kappa$_3$ opioid receptor and thereby competing with opioid binding thereto, (e) growing the hybridoma cell so
20 isolated under conditions permitting the production of the monoclonal antibody, and (f) recovering the monoclonal antibody so produced, thereby obtaining a purified monoclonal antibody capable of specifically binding to kappa$_3$ opioid receptor and thereby competing with opioid
25 binding thereto.

Methods of making hybridomas and monoclonal antibodies are well known to those skilled in the art.

30 The subject invention further provides the hybridoma cell produced in step (d) of the subject invention.

27

The subject invention further provides the purified
monoclonal antibody produced by the method of the subject
invention.

5   As used herein, a "purified monoclonal antibody" means the
monoclonal antibody free of any other antibodies.

The subject invention further provides an antibody capable
of specifically binding to kappa$_3$ opioid receptor and
10 thereby competing with opioid binding thereto, said antibody
being labeled with a detectable marker.

The labeled antibody may be a polyclonal or monoclonal
antibody.   In one embodiment, the labeled antibody is a
15 purified labeled antibody. The term "antibody" includes, by
way of example, both naturally occurring and non-naturally
occurring antibodies.   Specifically, the term "antibody"
includes polyclonal and monoclonal antibodies, and fragments
thereof. Furthermore, the term "antibody" includes chimeric
20 antibodies and wholly synthetic antibodies, and fragments
thereof.   The detectable marker may be, for example,
radioactive or fluorescent. Methods of labeling antibodies
are well known in the art.

25 The subject invention further provides a method for
detecting the presence of kappa$_3$ opioid receptor in a sample
which comprises contacting the sample with the antibody of
the subject invention under conditions permitting the
antibody to form a complex with kappa$_3$ opioid receptor
30 present in the sample, and detecting the presence of complex
so formed, so as to thereby detect the presence of kappa$_3$
opioid receptor in the sample.

28

The sample may be, for example, a cell sample, tissue sample, or protein-containing fluid sample. Conditions permitting the antibody to form a complex with kappa$_3$ opioid receptor and methods of detecting the presence of complex so
5 formed are well known in the art.

The subject invention further provides a method for quantitatively determining the amount of kappa$_3$ opioid receptor in a sample which comprises contacting the sample
10 with the antibody of the subject invention under conditions permitting the antibody to form a complex with kappa$_3$ opioid receptor present in the sample, quantitatively determining the amount of complex so formed, and comparing the amount so determined with a known standard, so as to thereby
15 quantitatively determine the amount of kappa$_3$ opioid receptor in the sample.

The subject invention further provides a composition which comprises the antibody of the subject invention in an amount
20 effective to permit imaging cell membrane-bound kappa$_3$ opioid receptor present in a subject, and a pharmaceutically acceptable carrier.

The amount effective to permit imaging cell membrane-bound
25 kappa$_3$ opioid receptor present in a subject may be calculated using methods well known in the art.

Pharmaceutically acceptable carriers are well known to those skilled in the art and include, but are not limited to,
30 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol,

vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media.

5 Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preserva-

10 tives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like.

The subject invention further provides a method for imaging
15 cell membrane-bound kappa$_3$ opioid receptor present in a subject which comprises administering to the subject an amount of the composition of the subject invention effective to permit imaging cell membrane-bound kappa$_3$ opioid receptor present in the subject under conditions permitting the

20 antibody of the composition to specifically bind to cell membrane-bound kappa$_3$ opioid receptor present in the subject, and imaging the antibody specifically bound to cell membrane-bound kappa$_3$ opioid receptor present in the subject after a suitable period of time, so as to thereby image cell

25 membrane-bound kappa$_3$ opioid receptor present in the subject.

In one embodiment, the subject is a mouse. In another embodiment, the subject is a human.

30
As used herein, "imaging" means determining the physical location of cell membrane-bound kappa$_3$ opioid receptor present in the subject. General methods of imaging cell membrane-bound proteins using antibody-based probes are well

known to those skilled in the art. The signal detected in the imaging method of the subject invention consists of signal from labeled antibody bound to cell membrane-bound kappa₃ opioid receptor. The amount of antibody may be

5   saturating or non-saturating. As used herein, "saturating" means that the number of receptor binding-sites on the antibodies exceeds the number of antibody-binding sites on the receptors.

10   Imaging the antibody specifically bound to cell membrane-bound kappa₃ opioid receptor is accomplished according to methods well known to those skilled in the art. Such methods include, by way of example, using a gamma camera to measure the signal emitted by the antibody bound to receptor

15   present in a subject. The imaging methods and quantitative methods of the subject invention can be combined, and means of doing so are well known in the art.

As used herein, a "suitable period of time" means a period

20   of time after which substantially all of the non-specifically bound antibody has be excreted from the subject, but by which a detectable amount of antibody remains bound to the receptor present in the subject.

25   The subject invention further provides a composition which comprises the antibody of the subject invention in an amount effective to permit quantitatively determining the amount of cell membrane-bound kappa₃ opioid receptor present in a subject, and a pharmaceutically acceptable carrier.

30

The amount effective to permit quantitatively determining the amount of cell membrane-bound kappa₃ opioid receptor can be determined according to methods well known in the art.

The subject invention further provides a method for quantitatively determining the amount of cell membrane-bound kappa₃ opioid receptor present in a subject which comprises administering to the subject an amount of the composition of

5 the subject invention effective to permit quantitatively determining the amount of cell membrane-bound kappa₃ opioid receptor present in the subject under conditions permitting the antibody of the composition to specifically bind to cell membrane-bound kappa₃ opioid receptor in the subject,

10 quantitatively determining the amount of antibody specifically bound to cell membrane-associated kappa₃ opioid receptors present in the subject, and comparing the amount so determined with a known standard so as to thereby quantitatively determine the amount of cell membrane-bound

15 kappa₃ opioid receptor present in the subject.

The amount of the composition effective to permit quantitatively determining the amount of receptor, conditions permitting the antibody to specifically bind to

20 kappa₃ opioid receptor, and methods of quantitatively determining the amount of antibody specifically bound to the receptors are well known in the art.

The amount of cell membrane-bound kappa₃ opioid receptor

25 need not be an absolute number, in the sense that it need not be the actual number of receptors in the subject. Rather, the amount may merely correlate with this number.

The methods of the subject invention for imaging and

30 quantitatively determining the amount of receptor in an entire subject may be analogously applied to individual organs in the subject.

32

The subject invention further provides a method for determining a subject's potential sensitivity to a kappa$_3$ opioid receptor-specific agent which comprises quantitatively determining the amount of cell membrane-bound

5   kappa$_3$ opioid receptor present in the subject by the method of the subject invention, and comparing the amount of cell membrane-bound kappa$_3$ opioid receptor so determined with the amount of cell membrane-bound kappa$_3$ opioid receptor present in a subject having a known sensitivity to the agent, so as

10   to thereby determine the subject's potential sensitivity to the kappa$_3$ opioid receptor-specific agent.

As used herein, a subject's potential sensitivity to a kappa$_3$ opioid receptor-specific agent is the degree to which

15   the agent is capable of eliciting a kappa$_3$ opioid receptor-mediated physiological response in the subject.

As used herein, the term "agent" includes both protein and non-protein moieties. For example, the agent may be an

20   antibody directed against a portion of kappa$_3$ opioid receptor, or a kappa$_3$ opioid or analog thereof.

In one embodiment, the kappa$_3$ opioid receptor-specific agent is a kappa$_3$ opioid receptor-specific analgesic agent.

25

The subject invention further provides a method for determining the affinity of an agent for kappa$_3$ opioid receptor which comprises (a) contacting a predetermined amount of kappa$_3$ opioid receptor with (i) a predetermined

30   amount of the agent together with (ii) a predetermined amount of a detectable known ligand of kappa$_3$ opioid receptor, said known ligand being a known ligand of kappa$_3$ opioid receptor with a known affinity therefore, under conditions which would permit the formation of a complex

between kappa$_3$ opioid receptor and the detectable known ligand in the absence of the agent, (b) quantitatively determining the amount of kappa$_3$ opioid receptor-detectable known ligand complex so formed, (c) comparing the amount of

5   kappa$_3$ opioid receptor-detectable known ligand complex determined in step (b) with the amount of kappa$_3$ opioid receptor-detectable known ligand complex formed in the absence of the agent, and (d) determining the affinity of the agent for kappa$_3$ opioid receptor based on the comparison

10 in step (c).

The detectable known ligand of kappa$_3$ opioid receptor may be, for example, radiolabeled, fluorescently labeled or enzymatically labeled.

15

Conditions permitting the formation of a complex, and methods for quantitatively determining the amount of receptor-ligand complex are well known in the art. Methods of determining the affinity of the agent for kappa$_3$ opioid

20 receptor based on the comparison in step (c) are also well known in the art.

The subject invention further provides a method for determining the affinity of an agent for kappa$_3$ opioid

25 receptor which comprises (a) contacting kappa$_3$ opioid receptor with the agent under conditions which would permit the formation of a complex between kappa$_3$ opioid receptor and a known ligand thereof, (b) contacting a predetermined amount of the agent-receptor complex with a predetermined

30 amount of the antibody of the subject invention under conditions which would permit the formation of a complex between kappa$_3$ opioid receptor and the antibody in the absence of the agent, (c) quantitatively determining the amount of kappa$_3$ opioid receptor-antibody complex so formed,

and (d) determining the affinity of the agent for kappa$_3$ opioid receptor based on the amount of kappa$_3$ opioid receptor-antibody complex determined in step (c).

5 The subject invention further provides a method for determining whether a known ligand of kappa$_3$ opioid receptor is an agonist thereof which comprises (a) contacting the kappa$_3$ opioid receptor with (i) a predetermined amount of the ligand and (ii) a predetermined amount of GTP or analog
10 thereof under conditions which would permit the binding of the ligand to the kappa$_3$ opioid receptor in the absence of GTP or analog thereof, (b) quantitatively determining the percentage of such predetermined amount of ligand bound to the kappa$_3$ opioid receptor, and (c) comparing the percentage
15 so determined with the percentage of such predetermined amount of ligand bound to the kappa$_3$ opioid receptor in the absence of GTP or analog thereof, a lower percentage in the presence of GTP or analog thereof relative to the percentage in the absence of GTP or analog thereof indicating that the
20 ligand is an agonist of kappa$_3$ opioid receptor.

The conditions, and methods of comparing, in this method are well known in the art.   GTP analogs include, by way of example, Gpp(NH$_2$)p and gamma-S-GTP.
25

The subject invention further provides a method for determining whether a known ligand of kappa$_3$ opioid receptor is an agonist thereof which comprises (a) contacting the kappa$_3$ opioid receptor with a predetermined amount of the
30 ligand under conditions which permit the binding of the ligand to the kappa$_3$ opioid receptor, (b) quantitatively determining the percentage of such predetermined amount of ligand bound to the kappa$_3$ opioid receptor, and (c) comparing the percentage to a known standard so as to

thereby determine whether the known ligand of kappa₃ opioid receptor is an agonist thereof.

The subject invention further provides a method for determining whether a ligand of kappa₃ opioid receptor is an antagonist thereof which comprises (a) contacting the kappa₃ opioid receptor with (i) a predetermined amount of the ligand and (ii) a predetermined amount of GTP or analog thereof under conditions which would permit the binding of the ligand to the kappa₃ opioid receptor in the absence of GTP or analog thereof, (b) quantitatively determining the percentage of such predetermined amount of ligand bound to the kappa₃ opioid receptor, and (c) comparing the percentage so determined with the percentage of such predetermined amount of ligand bound to the kappa₃ opioid receptor in the absence of GTP or analog thereof, an equality between the percentage in the presence of GTP or analog thereof and the percentage in the absence of GTP or analog thereof indicating that the ligand is an antagonist of kappa₃ opioid receptor.

The subject invention further provides a method for determining whether a known ligand of kappa₃ opioid receptor is an antagonist thereof which comprises (a) contacting the kappa₃ opioid receptor with a predetermined amount of the ligand under conditions which permit the binding of the ligand to the kappa₃ opioid receptor, (b) quantitatively determining the percentage of such predetermined amount of ligand bound to the kappa₃ opioid receptor, and (c) comparing the percentage to a known standard so as to thereby determine whether the known ligand of kappa₃ opioid receptor is an antagonist thereof.

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Finally, the subject invention provides kits for practicing the methods of the subject invention.

In order to facilitate an understanding of the Experimental
5   Details section which follows, certain frequently occurring methods and/or terms are best described in Sambrook, et al. (1989).

This invention will be better understood by reference to the
10 Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

I - Introduction

5

Opiate receptors mediate the actions of the opiates and opioid peptides. For many years, it has been known that a variety of opioid receptor subtypes exist. The first class of opiate receptors identified were termed mu and were based
10 upon the selectivity of drugs such as morphine. This classification was soon followed by additional binding sites called delta receptors, based upon enkephalin binding, and then kappa receptors, which are thought to mediate the actions of dynorphins. Recently multiple subtypes of kappa
15 receptors have been proposed (see, e.g., Pasternak, Clin. Neuropharmacol., 1993, for review and references). The $kappa_1$ receptor was initially defined in binding studies using drugs such as U50,488H and nor-binaltorphimine (norBNI). The $kappa_2$ receptor differs from the $kappa_1$
20 receptor in terms of the binding selectivity. Another kappa receptor subtype was identified which subtype is unique. Like the $kappa_2$ receptor, the $kappa_3$ receptors (Clark, et al. (1989), Price, et al. (1988), Gistrak, et al. (1989), and Paul, et al. (1989)) do not bind U50,488H or nor-
25 binaltorphimine very well. The overall binding selectivity of $kappa_3$ is quite different from that of the $kappa_2$ receptor as well as that of any other published receptor class. In addition to the unique binding profile, the $kappa_3$ receptors have a novel and distinctive pharmacology. Using a novel
30 ligand, naloxone benzoylhydrazone (NalBzoH), the ability of $kappa_3$ receptors to elicit analgesia was confirmed. This $kappa_3$ analgesia does not appear to be associated with respiratory depression or significant constipation. In this regard, the $kappa_3$ analgesia is quite distinct from the

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actions of morphine. Furthermore, antagonists which selectively block morphine (mu), delta and kappa$_1$ analagesia have little effect on kappa$_3$ analgesia. This observation is consistent with a unique site of action. Finally, the

5   continued administration of an opiate drug produces tolerance, i.e., a diminishing response to a repeated fixed dose of drug. Tolerance is seen with virtually all the opioid receptor classes. However, there is no cross-tolerance between kappa$_3$ receptors and the receptors of the

10 other classes. This absence of cross-tolerance implies that the mechanisms of both analgesia and tolerance associated with the kappa$_3$ receptor are distinct from the mechanisms of mu, delta and kappa$_1$ receptors. Based upon these observations, kappa$_3$ receptors represent a novel and unique

15 class of opioid receptor. However, validation of this concept requires the isolation and structural determination of the receptor, a membrane-bound protein. Described herein is the isolation of a cDNA clone encoding for a novel protein which appears to be the kappa$_3$ receptor.

20

II - **Cloning and Sequencing Strategy for Kappa$_3$ Opioid Receptor**

Two degenerate oligonucleotides were designed to encode the

25 predicted peptide sequences of the mouse δ receptor at peptide position 137-144 (sense orientation) and 312-319 (anti-sense orientation), which are highly conserved among the G protein-coupled receptor family. The oligonucleotides were used in PCR reactions to amplify mouse kappa$_3$ opioid

30 receptor cDNA from a mouse brain lambda ZAP cDNA library (Stratagene). The PCR amplification resulted in the production of an approximately 500bp fragment which was subcloned into the Bluescript SK(-) at the EcoRV site. The DNA sequences of the fragment were determined. The

nucleotide and predicted peptide sequences of the PCR fragment were highly homologous with those of the mouse δ receptor.

5   The PCR fragment was radiolabeled with $\alpha[32P]dCTP$ and used as a probe to screen the same mouse brain lambda ZAP cDNA library by hybridization. Approximately 2.8×10^6 plaques were probed at high stringency. Eighteen clones were isolated from this screening. The Bluescript SK(-) plasmids

10 containing inserts were then excised from these clones with super-infection of VCSM 13 helper bacteriophage and sizes were verified. One of the longest clones (13-2A4) contains an insert of approximately 2.6 kb. The entire clone was sequenced. The protein sequence is predicted to begin at

15 the first ATG at nucleotide number 96 in the sequence, preceded by two termination codons upstream at positions 7 and 72.

The amino acid sequence predicted from the cDNA encodes a

20 protein containing 367 residues with a calculated molecular weight of 41,047 daltons, and reveals seven hydrophobic domains.

Comparison of the protein sequence with published structures

25 from the opioid receptor family indicates an overall identity with MOR-1, KOR-1 and DOR-1 predicted protein sequences of 50.1%, 48% and 46%, respectively. However, these homology values are somewhat misleading. Portions of the molecule, especially the N- and C-termini, are

30 relatively unique, sharing little homology with the other cloned receptors. However, some of the transmembrane regions show a very close homology, with stretches of identity exceeding 20 amino acid residues in length at points.

40

III - Expression of Kappa₃ Opioid Receptor

The kappa₃ opioid receptor clone was expressed in COS-7
cells (Table 1).

Table 1

Expression of opioid binding in COS-7 cells
with kappa₃ opioid receptor

	3H-Diprenorphine Binding (cpm)
pSRα	789 ± 79
Control	157 ± 20

The XhoI/BamHI fragment of the clone was subcloned into a
pSRα expression vector at the XhoI/BamHI sites. COS-7 cells
were transfected with 30 μg of plasmid DNA/150mm plate using
DEAE-dextran. The transfected cells were grown at 37°C for
72 hours and the membranes were harvested for binding
assays. Control binding was determined using COS-7 cells
which were treated with DEAE-dextran and incubated under the
same conditions as the transfected cells. ³H-Diprenorphine
binding was determined in samples with 0.5 mg protein/tube.
All binding was assayed in triplicate, except for
nonspecific binding in the pSRα which was assayed in
duplicate. Only specific opioid binding (i.e., total minus
nonspecific) is presented. Results are the means ± s.e.m.
The pSRα results are significantly different from the
control COS-7 cells (p<0.001) as determined by the t-Test.

The XhoI/BamHI fragment of the clone was obtained and subcloned into a pSRα expression vector at the XhoI/BamHI sites and then transfected the COS-7 cells, which were assayed for opioid binding after 72 hours. To control for

5   the treatments, the binding in COS-7 cells was also examined, which cells were exposed to DEAE-dextran and incubated under the same conditions as the transfected cells. Additional studies using pCMV vectors and the same transfection techniques reveal binding values virtually

10 identical to those seen in this control (data not shown). Opioid binding was determined with the antagonist ^{3}H-diprenorphine. Binding is performed in the absence of a high concentration of active opioid (levallorphan) and specific binding defined as the difference in binding (i.e.,

15 the binding sensitive to the active opioid). ^{3}H-diprenorphine binding was observed following transfection with the clone which is significantly different from the control binding.

20 IV - **Association of Kappa₃ Opioid Receptor in Kappa3**
        Analgesia

    To determine the pharmacological relevance of the clone, an antisense oligodeoxynucleotide was designed and injected

25 intracerebroventricularly in mice as described in section V, *infra*. Controls consisted of saline vehicle, the complimentary sense oligodeoxynucleotide against the antisense oligodeoxynucleotide and a mismatch antisense oligodeoxynucleotide in which were switched two base pairs

30 from the antisense sequence. As shown in Figure 2, the antisense treatment markedly lowered the analgesic response to NalBzoH (p<0.002) without interfering with the response to morphine. The selectivity of the response was confirmed

by the lack of activity of the sense strand or the mismatched antisense probe.

V - Effect of antisense treatment on kappa₃ analgesic activity

Groups of mice received intracerebroventricular injections of 5 μg of either 1) anti-sense to the kappa₃ clone (5'-GGGCTGTGCAGAAGCGCAGA-3') (n=29 in the morphine group and n=29 in the NalBzoH group); 2) the complementary sense strand to this oligodeoxynucleotide (5'-CCCGACACGTCTTCGC-GTCT-3') (n=29 in the morphine group and n=29 in the NalBzoH group); or a mismatch antisense or saline on days 1, 3 and 5. On day 6, animals were tested for analgesia to either morphine (1 μg) or NalBzoH (15 μg). An additional group was treated identically with a mismatched antisense in which two base pairs had been switched (5'-GGGTCGTGCAGAGACGCAGA-3') (n=29) and tested only with NalBzoH. Analgesia was assessed quantally using the tailflick assay. In brief, a mouse tail is exposed to a hot beam of light and the duration of time the mouse permits the tail to remain in the beam of light is measured. Typical latencies range between 2 to 3 seconds. Fifteen minutes after the injection of drug, the test is repeated. Analgesia is defined quantally as a doubling or greater of the tailflick latency compared to the baseline determined for each individual mouse. Statistical significance is determined using the Fischer exact test. Morphine analgesia is unaffected by anti-sense treatment. NalBzoH analgesia is unaffected by the sense or the mismatched antisense treatments, but is markedly reduced by the antisense treatment ($p < 0.002$).

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VI - <u>Conclusion</u>

A clone was isolated and sequenced which appears to
5   correspond to the kappa$_3$ receptor initially proposed several
years ago. The clone was expressed in COS-7 cells, as
demonstrated by the appearance of opioid binding, indicating
that the protein was synthesized. Finally, a highly
specific antisense approach was utilized to demonstrate the
10 involvement of the protein encoded by this clone in kappa$_3$
analgesia, but not that of morphine induced analgesia.

References

Adelman et al. (1983) DNA 2:183.

5 Akil, H. et al. (1984) Annu. Rev. Neurosci. 7:223.

Attali, B. et al. (1989) J. Neurochem. 52:360.

Bertin, B. et al. (1992) J. Biol. Chem. 267(12):8200.
10
Bero, et al. (1988) Mol. Pharmacol. 34:614.

Bertolucci, M. et al. Neurosci, Abstr. 18L1368.

15 Bolivar et al., (1977) Gene, 2:95.

Boshart et al. (1985) Cell 41:521.

Bouvier, M. et al. (1988) Mol. Pharmacol. 33:133.
20
Bradbury, A. F. et al. (1976) Nature 260:165.

Breder, C. D. et al. (1992). J. Neurosci 12:3920.

25 Chang et al., (1978) Nature, 375:615.

Clark, J. A. et al. (1989) J. Pharmacol. Expt. Therapeut.
251:461.

30 Cotecchia et al. (1988) Proc. Natl. Acad. Sci. USA 85:7159.

Crea et al., (1978) Proc. Natl. Acad. Sci. U.S.A, 75:5765.

Danboldt, N.C. et al. (1990) Biochemistry 29(28):6734.
35

45

Di Chiara, G. et al. (1992) Trends Pharmacol. Sci. 13:185.

Dohlman (1987) Biochemistry 26:2657.

5   Dohlman, H.G. (1991) Annu. Rev. Biochem. 60:166-170; 174-
s176; 653-688.

Ferruti, P. and Tanzi, M.C., (1986) Cris. Rev. Ther. Drug
Carrier Syst. 2:117-136.

10
Fiers et al., (1978) Nature 273:113.

Frielle, T. et al. (1988) Proc. Natl. Acad. Sci. USA
85:9484.

15
Gabizon, A. et al., (1990) Cancer Res. 50:6371-6378

Gioannini, T. L. et al. (1989) J. Mol. Recogn. 2:44.

20   Goeddel et al., (1979) Nature, 281:544.

Goeddel et al., (1980) Nucleic Acids Res., 8:4057.

Gransch, C. et al. (1988) J. Biol. Chem. 263:5853.
25
Hess et al., (1968) J. Adv. Enzyme Reg. 7:149.

Hitzeman et al., (1980) J. Biol. Chem. 255:2073.

30   Holland et al., (1978) Biochemistry 17:4900.

Horstman, D. A. et al. (1990) J. Biol. Chem. 265:21590.

Hsia, J. A. et al. (1984) J. Biol. Chem. 259:1086.
35

Hughes, J. et al. (1975) Nature 258:577.

Itakura et al., (1977) Science, 198:1056.

5   Jones, (1977) Genetics 85:12.

Kanaho et al. (1984) J. Biol. Chem. 259:7378.

Kennelly, P. J. et al. (1991) J. Biol. Chem. 266:15555.
10
King et al. (1990) Science 250:121.

Kingsman et al., (1979) Gene 7:141.

15   Kobilka, B. K. et al. (1987) J. Biol. Chem. 262:7321.

Kobilka, B. K. et al. (1988) Science 240:1310.

Koob, G. F. et al. (1992) Trends Neurosci. 15:186.
20
Kozasa et al. (1988) Proc. Natl. Acad. Sci USA 85:2081.

Kruse and Patterson, eds. (1973) Tissue Culture, Academic
Press.
25
Kyte, J., and R.F. Doolittle (1982) J. Mol. Biol. 157:105.

Loh, H. H. et al., (1990) Annu. Rev. Pharmacol. Toxicol.
30:123.
30
Loh, H. H. et al. (1990) Annu. Rev. Pharmacol. Toxicol.
30:123.

Lomasney et al. (1990) Proc. Natl. Acad. Sci. USA 87:5094.
35

47

Lutz, R. A. et al. (1992) J. Receptor Res. 12:267.

Mansour, A. et al. (1987) J. Neurosci. 7:2445.

5 Marullo et al. (1988) Proc. Natl. Acad. Sci. USA 85:7551.

Messing et al., Third Cleveland Symposium on Macromolecules and Recombinant DNA, Editor A. Walton, Elsevier, Amsterdam (1981).

10

Nathans et al. (1986 A) Science 232:193.

Nathans et al. (1986 B) Science 232:203

15 Nock, B. et al. (1988) Eur. J. Pharmacol. 154:27.

Okayama et al. (1983) Mol. Cell Biol. 3:280.

Olson, G. A. et al. (1989) Peptides 10:1253.

20

Ott, S. et al. (1988) J. Biol. Chem. 263:10524.

Payette et al. (1990) FEBS Lett. 266:21.

25 Pert, C. G. et al. (1973) Science 179:1011.

Pert, C. B. et al. (1974) Mol. Pharmacol. 10:868.

Pfeiffer, A. et al. (1986) Science 223:774.

30

Puttfarcken, P. S. et al. (1988) Mol. Pharmacol. 33:520.

Ranade, V. V. (1989) J. Clin. Pharmacol. 29:685-694

35 Regan et al. (1988) Proc. Natl. Acad. Sci. USA 85:6301.

48

Sambrook, J. et al. (1989) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

5 Seeburg (1982) DNA 1:239.

Shook, J. E. et al. (1990) Am. Rev. Respir. Dis. 142:895.

Siebwenlist et al., (1980) Cell, 20:269.
10
Simon, E. J. (1991) Medicinal Res. Rev. 11:357.

Stinchcomb et al., (1979) Nature, 282:39.

15 Stratford-Perricaudet et al. (1992).

Strotchman and Simon (1991).

Thomsen et al. (1984) PNAS 81:659.
20
Tschemper et al., (1980) Gene 10:157.

Unterwald, E. M. et al. (1991) Brain Res. 562:57.

25 Unterwald, E. M. et al. (1987) Eur. J. Pharmacol. 133:275.

Xie, G-X., et al. (1992) Proc. Natl. Acad. Sci. USA 89:4124.

Yamada, Y. et al. (1992) Proc. Natl. Acad. Sci. USA 89:251.
30
Yasuda, K. et al. (1992) J. Biol Chem. 267:20422.

Yokota, Y. et al. (1992) EMBO J. 11:3585.

Zukin, R. S. et al. (1988) Proc. Natl. Acad. Sci. USA 85:4061.

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What is claimed is:

1.   A recombinant nucleic acid molecule which encodes a
5   kappa₃ opioid receptor.

2.   The recombinant nucleic acid molecule of claim 1,
        wherein the nucleic acid molecule is a DNA molecule.

10 3.   The recombinant nucleic acid molecule of claim 2,
        wherein the DNA molecule is a cDNA molecule.

4.   The recombinant nucleic acid molecule of claim 1,
        wherein the nucleic acid molecule is an RNA molecule.
15
5.   The recombinant nucleic acid molecule of claim 1,
        wherein the kappa₃ opioid receptor is native kappa₃
        opioid receptor.

20 6.   The recombinant nucleic acid molecule of claim 1,
        wherein the kappa₃ opioid receptor is a variant kappa₃
        opioid receptor.

7.   The recombinant nucleic acid molecule of claim 6,
25   wherein the variant kappa₃ opioid receptor is an
        aqueous-soluble variant kappa₃ opioid receptor.

8.   The recombinant nucleic acid molecule of claim 1,
        wherein the kappa₃ opioid receptor is a mammalian kappa₃
30   opioid receptor.

9.   The recombinant nucleic acid molecule of claim 8,
        wherein the mammalian kappa₃ opioid receptor is a
        murine kappa₃ opioid receptor.

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10. The recombinant nucleic acid molecule of claim 8, wherein the mammalian kappa$_3$ opioid receptor is a human kappa$_3$ opioid receptor.

5   11. An anti-sense oligonucleotide molecule capable of specifically hybridizing to an mRNA molecule encoding kappa$_3$ opioid receptor, at the portion thereof encoding kappa$_3$ opioid receptor, so as to prevent translation of the mRNA molecule.

10

12. A nucleic acid molecule encoding the anti-sense oligonucleotide molecule of claim 11, wherein the nucleic acid molecule is capable of being expressed in a suitable host cell.

15

13. A vector comprising the recombinant nucleic acid molecule of claim 3.

14. The vector of claim 13, wherein the vector is a
20   plasmid.

15. The plasmid of claim 14, wherein the plasmid is designated 13-2A4 (ATCC Accession No. 75608).

25   16. A host vector system for the production of a kappa$_3$ opioid receptor which comprises the vector of claim 13 in a suitable host cell.

17. The host vector system of claim 16, wherein the
30   suitable host cell is a bacterial cell.

18. The host vector system of claim 17, wherein the bacterial cell is an E. coli cell.

19. The host vector system of claim 16, wherein the suitable host cell is an eucaryotic cell.

20. The host vector system of claim 19, wherein the eucaryotic cell is a mammalian cell.

21. The host vector system of claim 20, wherein the mammalian cell is a COS cell.

22. The host vector system of claim 21, wherein the COS cell is a COS-7 cell.

23. A method for producing a kappa$_3$ opioid receptor which comprises growing the host vector system of claim 16 under conditions permitting the production of the kappa$_3$ opioid receptor, and recovering the kappa$_3$ opioid receptor produced thereby.

24. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule is labeled with a detectable marker.

25. A method for detecting the presence of kappa$_3$ opioid receptor-encoding nucleic acid molecules present in a sample which comprises contacting the sample with the labeled nucleic acid molecule of claim 24 under conditions permitting the labeled nucleic acid molecule to form a complex with kappa$_3$ opioid receptor-encoding nucleic acid molecules present in the sample, and detecting the presence of such complex formed so as to thereby detect the presence of kappa$_3$ opioid receptor-encoding nucleic acid molecules present in the sample.

53

26. A method for quantitatively determining the amount of kappa₃ opioid receptor-encoding nucleic acid molecules present in a sample which comprises contacting the sample with a suitable amount of the labeled nucleic acid molecule of claim 24 under conditions permitting the labeled nucleic acid molecule to form a complex with kappa₃ opioid receptor-encoding nucleic acid molecules present in the sample, quantitatively determining the amount of complex so formed so as to thereby quantitatively determine the amount of kappa₃ opioid receptor-encoding nucleic acid molecules present in the sample.

27. A purified native kappa₃ opioid receptor.

28. The variant kappa₃ opioid receptor encoded by the recombinant nucleic acid molecule of claim 6.

29. A method for obtaining partially purified polyclonal antibodies capable of specifically binding to kappa₃ opioid receptor and thereby competing with opioid binding thereto which method comprises (a) immunizing a subject with the kappa₃ opioid receptor encoded by the nucleic acid molecule of claim 1, (b) recovering from the immunized subject serum comprising antibodies capable of specifically binding to kappa₃ opioid receptor and thereby competing with opioid binding thereto, and (c) partially purifying the antibodies present in the serum, thereby obtaining partially purified polyclonal antibodies capable of specifically binding to kappa₃ opioid receptor and thereby competing with opioid binding thereto.

30.   The partially purified antibodies produced by the
       method of claim 29.

31.   A method for obtaining a purified monoclonal antibody
5   capable of specifically binding to kappa₃ opioid
       receptor and thereby competing with opioid binding
       thereto which method comprises (a) immunizing a subject
       with the kappa₃ opioid receptor encoded by the nucleic
       acid molecule of claim 1, (b) recovering from the
10   immunized subject a B cell-containing cell sample, (c)
       contacting the B cell-containing cell sample so
       recovered with myeloma cells under conditions
       permitting fusion of the myeloma cells with the B cells
       therein so as to form hybridoma cells, (d) isolating
15   from the resulting sample a hybridoma cell capable of
       producing a monoclonal antibody capable of specifically
       binding to kappa₃ opioid receptor and thereby competing
       with opioid binding thereto, (e) growing the hybridoma
       cell so isolated under conditions permitting the
20   production of the monoclonal antibody, and (f)
       recovering the monoclonal antibody so produced, thereby
       obtaining a purified monoclonal antibody capable of
       specifically binding to kappa₃ opioid receptor and
       thereby competing with opioid binding thereto.
25
32.   The hybridoma cell produced in step (d) of claim 31.

33.   The purified monoclonal antibody produced by the method
       of claim 31.
30
34.   An antibody capable of specifically binding to kappa₃
       opioid receptor and thereby competing with opioid
       binding thereto, said antibody being labeled with a
       detectable marker.

35. A method for detecting the presence of kappa$_3$ opioid receptor in a sample which comprises contacting the sample with the antibody of claim 34 under conditions permitting the antibody to form a complex with kappa$_3$

5   opioid receptor present in the sample, and detecting the presence of complex so formed, so as to thereby detect the presence of kappa$_3$ opioid receptor in the sample.

10 36. A method for quantitatively determining the amount of kappa$_3$ opioid receptor in a sample which comprises contacting the sample with the antibody of claim 34 under conditions permitting the antibody to form a complex with kappa$_3$ opioid receptor present in the

15   sample, quantitatively determining the amount of complex so formed, and comparing the amount so determined with a known standard, so as to thereby quantitatively determine the amount of kappa$_3$ opioid receptor in the sample.

20
37. A composition which comprises the antibody of claim 34 in an amount effective to permit imaging cell membrane-bound kappa$_3$ opioid receptor present in a subject, and a pharmaceutically acceptable carrier.

25
38. A method for imaging cell membrane-bound kappa$_3$ opioid receptor present in a subject which comprises administering to the subject an amount of the composition of claim 37 effective to permit imaging

30   cell membrane-bound kappa$_3$ opioid receptor present in the subject under conditions permitting the antibody of the composition to specifically bind to cell membrane-bound kappa$_3$ opioid receptor present in the subject, and imaging the antibody specifically bound to cell

membrane-bound kappa₃ opioid receptor present in the
subject after a suitable period of time, so as to
thereby image cell membrane-bound kappa₃ opioid
receptor present in the subject.

5

39. A composition which comprises the antibody of claim 34
     in an amount effective to permit quantitatively
     determining the amount of cell membrane-bound kappa₃
     opioid receptor present in a subject, and a
10   pharmaceutically acceptable carrier.

40. A method for quantitatively determining the amount of
     cell membrane-bound kappa₃ opioid receptor present in
     a subject which comprises administering to the subject
15   an amount of the composition of claim 39 effective to
     permit quantitatively determining the amount of cell
     membrane-bound kappa₃ opioid receptor present in the
     subject under conditions permitting the antibody of the
     composition to specifically bind to cell membrane-bound
20   kappa₃ opioid receptor in the subject, quantitatively
     determining the amount of antibody specifically bound
     to cell membrane-associated kappa₃ opioid receptors
     present in the subject, and comparing the amount so
     determined with a known standard so as to thereby
25   quantitatively determine the amount of cell membrane-
     bound kappa₃ opioid receptor present in the subject.

41. A method for determining a subject's potential
     sensitivity to a kappa₃ opioid receptor-specific agent
30   which comprises quantitatively determining the amount
     of cell membrane-bound kappa₃ opioid receptor present
     in the subject by the method of claim 40, and comparing
     the amount of cell membrane-bound kappa₃ opioid
     receptor so determined with the amount of cell

membrane-bound kappa₃ opioid receptor present in a
subject having a known sensitivity to the agent, so as
to thereby determine the subject's potential
sensitivity to the kappa₃ opioid receptor-specific
5   agent.

42.   The method of claim 41, wherein the kappa₃ opioid
      receptor-specific agent is a kappa₃ opioid receptor-
      specific analgesic agent.
10

43.   A method for determining the affinity of an agent for
      kappa₃ opioid receptor which comprises (a) contacting
      a predetermined amount of kappa₃ opioid receptor with
      (i) a predetermined amount of the agent together with
15   (ii) a predetermined amount of a detectable known
      ligand of kappa₃ opioid receptor, said known ligand
      being a known ligand of kappa₃ opioid receptor with a
      known affinity therefore, under conditions which would
      permit the formation of a complex between kappa₃ opioid
20   receptor and the detectable known ligand in the absence
      of the agent, (b) quantitatively determining the amount
      of kappa₃ opioid receptor-detectable known ligand
      complex so formed, (c) comparing the amount of kappa₃
      opioid receptor-detectable known ligand complex
25   determined in step (b) with the amount of kappa₃ opioid
      receptor-detectable known ligand complex formed in the
      absence of the agent, and (d) determining the affinity
      of the agent for kappa₃ opioid receptor based on the
      comparison in step (c).
30

44.   A method for determining the affinity of an agent for
      kappa₃ opioid receptor which comprises (a) contacting
      kappa₃ opioid receptor with the agent under conditions
      which would permit the formation of a complex between

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kappa$_3$ opioid receptor and a known ligand thereof, (b) contacting a predetermined amount of the agent-receptor complex with a predetermined amount of the antibody of claim 34 under conditions which would permit the formation of a complex between kappa$_3$ opioid receptor and the antibody in the absence of the agent, (c) quantitatively determining the amount of kappa$_3$ opioid receptor-antibody complex so formed, and (d) determining the affinity of the agent for kappa$_3$ opioid receptor based on the amount of kappa$_3$ opioid receptor-antibody complex determined in step (c).

45. A method for determining whether a known ligand of kappa$_3$ opioid receptor is an agonist thereof which comprises (a) contacting the kappa$_3$ opioid receptor with (i) a predetermined amount of the ligand and (ii) a predetermined amount of GTP or analog thereof under conditions which would permit the binding of the ligand to the kappa$_3$ opioid receptor in the absence of GTP or analog thereof, (b) quantitatively determining the percentage of such predetermined amount of ligand bound to the kappa$_3$ opioid receptor, and (c) comparing the percentage so determined with the percentage of such predetermined amount of ligand bound to the kappa$_3$ opioid receptor in the absence of GTP or analog thereof, a lower percentage in the presence of GTP or analog thereof relative to the percentage in the absence of GTP or analog thereof indicating that the ligand is an agonist of kappa$_3$ opioid receptor.

46. A method for determining whether a ligand of kappa$_3$ opioid receptor is an antagonist thereof which comprises (a) contacting the kappa$_3$ opioid receptor with (i) a predetermined amount of the ligand and (ii)

a predetermined amount of GTP or analog thereof under conditions which would permit the binding of the ligand to the kappa₃ opioid receptor in the absence of GTP or analog thereof, (b) quantitatively determining the

5   percentage of such predetermined amount of ligand bound to the kappa₃ opioid receptor, and (c) comparing the percentage so determined with the percentage of such predetermined amount of ligand bound to the kappa₃ opioid receptor in the absence of GTP or analog

10   thereof, an equality between the percentage in the presence of GTP or analog thereof and the percentage in the absence of GTP or analog thereof indicating that the ligand is an antagonist of kappa₃ opioid receptor.

FIGURE 1-1

```
         10        20        30        40        50        60
TTTGGCTTCCTTCTCCAACCTGCGCAGCCCCTCCTTCTCTCAGCCGCAGCCTTCTGCCCC

         70        80        90       100       110       120
TCCCCCTTCTGGCTGCCGCACTGGCTGCTGCGTCTAGTCAATATCTTATCTTCGGAGCAG

        130       140       150       160       170       180
GAGCTAGGAGCCATTCCCAGCCGGAGCAGACCCCAAGCTAGAGTGAGAAGCATTACTCAG

        190       200       210       220       230       240
TTCATTGTGCTCCTGCCTGCCTTTCTGCTAAGCATTAGGGTCTGTTTTGGCCCAGCTTCT

        250       260       270       280       290       300
GAAGAGGTTGTGTGTGCTGTTGGAGGAACTGTACTGAGTGGCTTTGCAGGGTGACAGCAT
                                                          — M

        310       320       330       340       350       360
GGAGTCCCTCTTTCCTGCCCCATTCTGGGAGGTCTTGTATGGCAGCCACTTTCAAGGGAA
  E   S   L   F   P   A   P   F   W   E   V   L   Y   G   S   H   F   Q   G   N

        370       380       390       400       410       420
CCTGTCTCTCCTAAATGAGACCGTACCCCATCACCTGCTCCTCAATGCTAGCCACAGTGC
  L   S   L   L   N   E   T   V   P   H   H   L   L   L   N   A   S   H   S   A

        430       440       450       460       470       480
CTTCCTGCCCCTTGGACTCAAGGTCACCATCGTGGGGCTCTACTTGGCTGTGTGCATCGG
  F   L   P   L   G   L   K   V   T   I   V   G   L   Y   L   A   V   C   I   G

        490       500       510       520       530       540
GGGGCTCCTGGGGAACTGCCTCGTCATGTATGTCATCCTCAGGCACACCAAGATGAAGAC
  G   L   L   G   N   C   L   V   M   Y   V   I   L   R   H   T   K   M   K   T

        550       560       570       580       590       600
TGCTACCAACATTTACATATTTAATCTGGCACTGGCTGATACCCTGGTCTTGCTGACACT
  A   T   N   I   Y   I   F   N   L   A   L   A   D   T   L   V   L   L   T   L

        610       620       630       640       650       660
GCCCTTCCAGGGCACAGACATCCTTCTGGGCTTCTGGCCATTTGGGAATGCACTGTGCAA
  P   F   Q   G   T   D   I   L   L   G   F   W   P   F   G   N   A   L   C   K

        670       680       690       700       710       720
GACGGTCATTGCTATCGACTACTACAACATGTTTACCAGCACTTTCACTTTGACTGCCAT
  T   V   I   A   I   D   Y   Y   N   M   F   T   S   T   F   T   L   T   A   M

        730       740       750       760       770       780
GAGTGTAGACCGTTATGTAGCTATCTGCCACCCTATCCGTGCCCTTGATGTTCGGACATC
  S   V   D   R   Y   V   A   I   C   H   P   I   R   A   L   D   V   R   T   S
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FIGURE 1-2

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          790        800        810        820        830        840
CAGTAAAGCCCAGGCCGTTAATGTGGCCATATGGGCCTGGCTTCGGTGGTTGGTGTTCCT
  S   K   A   Q   A   V   N   V   A   I   W   A   W   L   R   W   L   V   F   L

          850        860        870        880        890        900
GTTGCCATCATGGGCTCAGCACAAGTGGAGGATGAAGAGATCGAGTGCCTGGTGGAGATC
  L   P   S   W   A   Q   H   K   W   R   M   K   R   S   S   A   W   W   R   S

          910        920        930        940        950        960
CCCGCCCCTCAGGACTATTGGGGCCCTGTATTTGCCATCTGCATCTTCCTTTTTTCCTTC
  P   P   L   R   T   I   G   A   L   Y   L   P   S   A   S   S   F   F   P   S

          970        980        990       1000       1010       1020
ATCATCCCGGTTACTGATCATCTCTGTCTGCTACAGCCTCATGATTCGACGACTTCGTGG
  S   S   R   L   L   I   I   S   V   C   Y   S   L   M   I   R   R   L   R   G

         1030       1040       1050       1060       1070       1080
TGTCCGGCTGCTTTCAGGCTCCCGAGAGAAGGACCGGAACCTGCGACGCATCACACGGCT
  V   R   L   L   S   G   S   R   E   K   D   R   N   L   R   R   I   T   R   L

         1090       1100       1110       1120       1130       1140
GGTACTGGTAGTTGTGGCTGTGTTTGTGGGCTGCTGGACACCTGTGCAGGTCTTTGTCCT
  V   L   V   V   V   A   V   F   V   G   C   W   T   P   V   Q   V   F   V   L

         1150       1160       1170       1180       1190       1200
GGTTCAAGGACTGGGTGTTCAGCCAGGTAGTGAGACTGCAGTAGCCATTCTGCGCTTCTG
  V   Q   G   L   G   V   Q   P   G   S   E   T   A   V   A   I   L   R   F   C

         1210       1220       1230       1240       1250       1260
CACAGCCCTGGGCTATGTCAACAGTTGTCTCAATCCCATTCTCTATGCTTTCTTGGATGA
  T   A   L   G   Y   V   N   S   C   L   N   P   I   L   Y   A   F   L   D   E

         1270       1280       1290       1300       1310       1320
GAACTTCAAGGCCTGCTTTAGAAAGTTCTGCTGTGCTTCTGCCCTGCACCGGGAGATGCA
  N   F   K   A   C   F   R   K   F   C   C   A   S   A   L   H   R   E   M   Q

         1330       1340       1350       1360       1370       1380
GGTTTCTGATCGTGTGCGCACAGTTGCCAAGGATGTAGGCCTTGGTTGCAAGACCTCTGA
  V   S   D   R   V   R   T   V   A   K   D   V   G   L   G   C   K   T   S   E

         1390       1400       1410       1420       1430       1440
GACAGTACCACGGCCGGCATGACTAGGCGTGGACCTGCCCATGGTGCCTGTCAGTCCTAG
  T   V   P   R   P   A

         1450       1460       1470       1480       1490       1500
AGGAAGACCTTTTAGCACCATGGGACAGGTCAAAGCATCAAGGTGGCCTCCATGGCTCTG

         1510       1520       1530       1540       1550       1560
TCAGATTAAGTTTCCTCCCTGGTATAGGACCAGAGAGAACCAAAGGAACTGCATGGAAAC
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FIGURE 1-3

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        1570      1580      1590      1600      1610      1620
ATCCACAACTCAGTGGACATGCCTGGTGAACCCATGTAGGTATTCATGGTTCACTTGACT

        1630      1640      1650      1660      1670      1680
CTTCTCTGGTTTCTCCCTGCTGCCCTGGTTCTAGGTGGGCTCAGCTGAGGTATTGTAGTT

        1690      1700      1710      1720      1730      1740
GTCATGTAGTCACTATTGTGACTACCTGTTGTGTGCTATTGCCCTCAGCCTTCAGTGTTT

        1750      1760      1770      1780      1790      1800
GCACAGAACTGGTGATCATACCCAGTGTTGCCTGGCCCTTAAGCTTGGAGTTGCCTTGGA

        1810      1820      1830      1840      1850      1860
GCATCTAGTTCTGACTCCACTGATGCATTCAGATTACCTGAGGTGGGTGAGCATCAGTGG

        1870      1880      1890      1900      1910      1920
GTTCTTGGATGACTGTTTCCTGACGATTCTTTTCATGCTGTACTATGGTGTATATGAAGG

        1930      1940      1950      1960      1970      1980
GGACTTCACACTTCATCTGGTACTGCCACTGCCTGCTCTACCAACCTGGACCACCTTCTC

        1990      2000      2010      2020      2030      2040
AGCAAGAGGCTAGCAGGGGGACAAGACACAAAGCTTCCCTAAGGCTCTTTCCCTCCAAAA

        2050      2060      2070      2080      2090      2100
CCACTGTGAACTCTTATTCTACAGACTGTTTGGCAAGCCCTGCTTCTAACTGTGTGGGAA

        2110      2120      2130      2140      2150      2160
GTAATCAGGAGAAAATTCTGTGGCCTCTGTAGGCTGCTCACAGCATGGAGGCACCACATG

        2170      2180      2190      2200      2210      2220
CTGGTCTTGGGTATGTGTCTTGGCTGCTCAGTATGGGCAGGGCAGGGCACGAGACTATCT

        2230      2240      2250      2260      2270      2280
CTCTCCTTATTCTCCACAGCCTCCCTCAGCTCTCCAGCAGTCGCTCTTTTACTTGACAGT

        2290      2300      2310      2320      2330      2340
AGAGGTTAGCAGCAGTTGTACTCGTAGAAACACACTTGTAGCCCGGGAAGACTGGAGTCA

        2350      2360      2370      2380      2390      2400
GGATGTGTTCTATTCTATACCCACAGTGACCACCTGCTTCATTTATAGGGTTAGGACATA

        2410      2420      2430      2440      2450      2460
TCCAAGCAAGGCCTGGGCTTGGCATCAAATGAAGAGCTGGTATGAGAGCTGAAGCCTAAA

        2470      2480      2490      2500      2510      2520
ATGGCTCATTTGAGCAATCTGCAAGGACTATTACGGTTTTGGGGACATTGGAAGAAGAGT
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FIGURE 1-4

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           2530        2540        2550          2560        2570        2580
CGATACCTTGGAGATATATTGTTGGTTCACAGAAGAAGAGGCTTTGTAAATGCCCTTTCT

           2590        2600        2610        2620        2630        2640
ATGGGTCAGATAAAAAAAAA
```
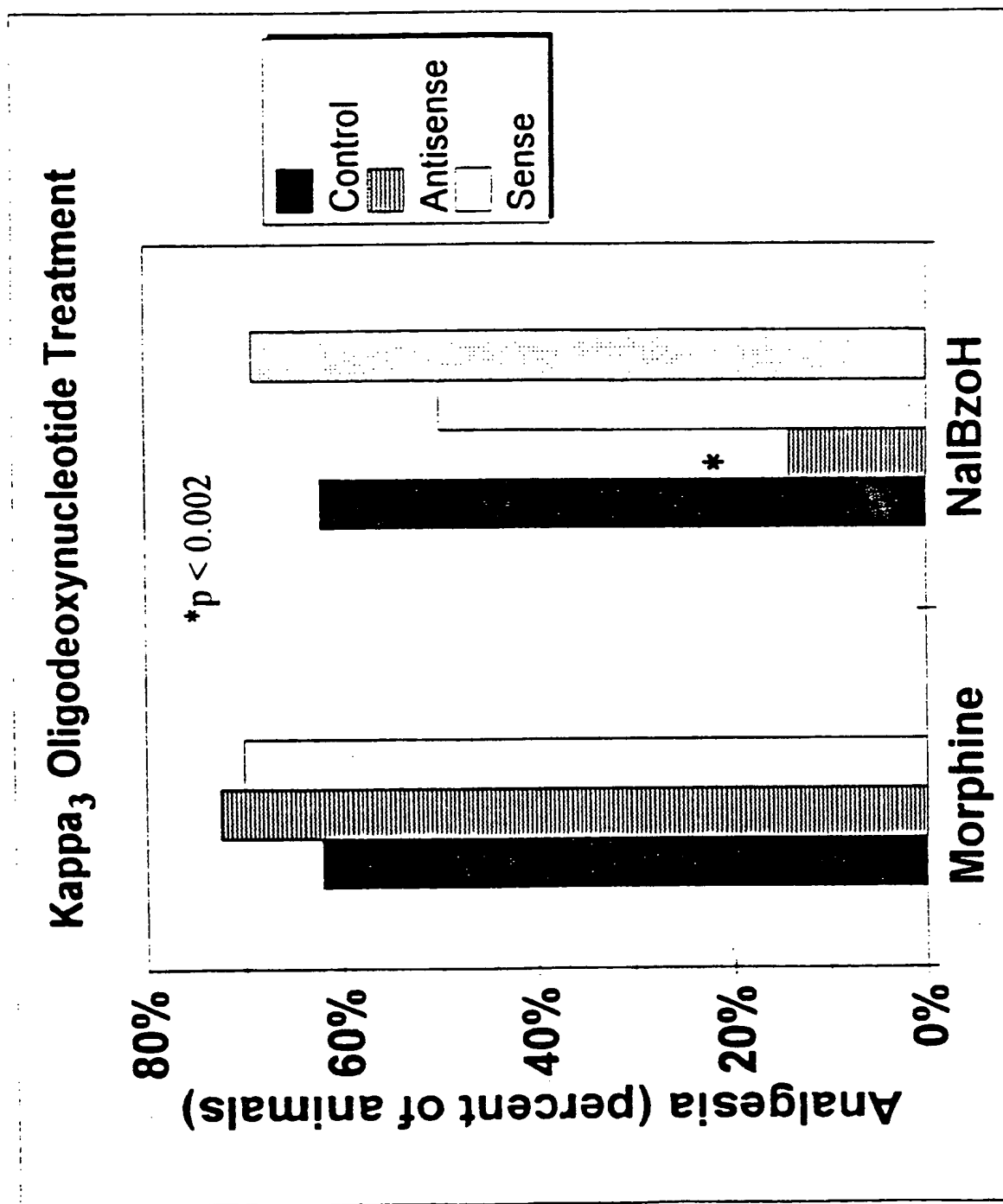
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/705, 16/00; C12N 5/10, 15/09, 15/63; C12Q 1/00
US CL :435/6, 7.1, 69.1, 240.1, 320.1; 530/350, 387.1, 395; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 69.1, 240.1, 320.1; 530/350, 387.1, 395; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Medline, Biosis, WPI
search terms: kappa3 opioid receptor, cloning, recombinant production, agonist antagonist, antibodies

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
Y	Federation of European Biochemical Societies, Volume 347, issued 1994, Chen et al, "Molecular Cloning, Tissue Distribution and Chromosomal Localization of a Novel Member of the Opioid Receptor Gene Family", pages 279-293, see pages 280-283.	1-46
Y	Biochemical Journal, Volume 295, Part 3, issued 01 November 1993, Chen et al, "Molecular Cloning of a Rat κOpioid Receptor Reveals Sequence Similarities to the μ and δ Opioid receptors", pages 625-628, see pages 626-628.	1-46

[X] Further documents are listed in the continuation of Box C.   [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 FEBRUARY 1995	09 FEB 1995

Name and mailing address of the ISA/US	Authorized officer
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INTERNATIONAL SEARCH REPORT

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proceedings of the National Academy of Sciences, Volume 85, issued October 1988, Goldstein et al, "'DAKLI': A Multipurpose Ligand with High Affinity and Selectivity for Dynorphin (κOpioid) Binding Sites", pages 7375-7379, see pages 7377-7378.	1-46
Y	Proceedings of the National Acadmy of Sciences, Volume 90, Number 21, issued 01 November 1993, Meng et al, "Cloning and Pharmacological Characterization of a Rat κ Opioid Receptor", Pages 9954-9958, see pages 9955-9957.	1-46
Y	Federation of European Biochemical Societies, Volume 348, issued 1994, Wang et al, "cDNA Cloning of an Orphan Opiate Receptor Gene Family Member and its Splice Variant", pages 75-79, see page 77.	1-46